

Isolation of an agent of the spotted fever group rickettsia from tick eggs in Madrid, Spain

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SUMMARY

Ticks recovered from dogs in rural villages around Madrid (Spain) were processed to isolate rickettsiae. One sample containing mixtures of ticks and four containing eggs, in which rickettsiae had been detected by indirect immunofluorescence with a human serum highly reactive to *Rickettsia conorii*, were decontaminated, homogenized and inoculated onto Vero cells. Two egg samples yielded a cytopathic agent that reacted positively by immunofluorescence. One sample (14H) was successfully subcultured and identified as a member of the spotted fever group rickettsia. Tick eggs provide suitable material for isolation of rickettsia.

Rickettsia conorii, the causative agent of Boutonneuse Fever, occurs in several countries of South Europe, Africa and Asia. In Spain cases have been reported in northern areas, the Mediterranean coast and central regions from 1982 [1–3], although cultivation of isolates has been described only recently [4]. The presence of rickettsiae of the spotted fever group (SFG) has been demonstrated in tick samples (*Rhipicephalus sanguineus*) from Spanish dogs, and antibody to rickettsiae in dog sera has been detected by indirect immunofluorescence (IF) [5] with *R. conorii* antigen. This shows the importance of the circulation of SFG rickettsiae in our region of Spain. This paper describes attempts to isolate rickettsiae from tick material and the recovery of one such agent from tick eggs.

During June and July 1988, 1139 ticks were recovered from 161 dogs in 15 different villages of the centre of Spain. The maintenance, processing and preliminary studies of these samples by IF with a human serum highly reactive to *R. conorii* have been described already [5]. Briefly, ticks and eggs delivered by them were grouped in 68 pools; 28 were positive for antigen by IF. Five of these IF positive samples (1 from ticks, 4 from eggs), all of which contained > 10 rickettsiae/microscopic field were selected for this work. They were all collected from villages of the province of Madrid.

Each suspension was thawed, 0.1 ml diluted 1/10 in Brain Heart Infusion broth (BHI) with NAD (0.38 mM) and ATP (2 mM) [6], inoculated onto Vero cells with

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Table 1. *Detection of rickettsiae by culture and immunofluorescence*

Specimen	Source	Ticks/eggs IF*	Cultures		
			CPE†	IF	Cont.‡
40	Ticks	15	—	—	+
14H	Eggs	30	+++	+	—
52H	Eggs	10	—	—	+
33H	Eggs	20	—	—	+
15H	Eggs	10	++	+	—

* Number of bacilli per field in the inoculum.

† Cytopathic effect after 2–5 days.

‡ Contamination.

Minimal Essential Medium (MEM) and Fetal Bovine Serum (FBS) without antibiotics and incubated at 32–33 °C for 2 h with gentle agitation. The medium was then replaced by the isolation medium containing MEM, FBS (10%), non-essential aminoacids (1%) and glutamine (1%). Cultures were incubated at 32 °C in 5% CO₂ and observed daily under the microscope, and aliquots of them taken for immunofluorescence studies.

Rickettsiae were detected in original material and Vero cells by IF, using a human serum highly reactive for *R. conorii* from our diagnostic laboratory, and a fluorescein-labelled antihuman conjugate (DAKO). *R. conorii* antigen from the Center for Disease Control (CDC, Atlanta, Georgia, USA) and a negative human serum were used as controls.

The number of rickettsiae in the original materials varied between 10 and 30 per field (Table 1). Samples 14H and 15H produced a cytopathic effect in Vero cells and gave a positive IF result. Sample 14H was successfully subcultured up to passage 19, yielding an isolate that reacted positively by IF in all passages. A sample of culture 14H was sent to CDC Atlanta where it was identified as *R. rhipicephali* by DNA analysis (R. Regnery, pers comm). Sample 15H could not be subcultured after the first passage. The other three cultures did not produce any cytopathic effect and were negative by IF; they were discontinued because of bacterial contamination.

Four of the five samples selected for this study contained tick eggs which are probably cleaner and less contaminated than ticks, and so more suitable as starting material and easier to work with for isolation attempts.

A human serum reactive with *R. conorii* does not specifically identify the organism as *R. conorii*, because wide cross reactivity occurs in rickettsia belonging to the SFG [7]. For this reason we sent a sample of isolate 14H to the rickettsial laboratory at CDC, where it was identified as *R. rhipicephali*.

R. rhipicephali is considered to be non-pathogenic for humans. However, its presence in Spanish dogs may be relevant in the interpretation of seroprevalence studies, because this organism can elicit antibodies cross-reactive for *R. conorii*. It is also possible that the two species may cross-protect in dogs. This could be due to the presence of a common polysaccharide antigen [8], although serological differences have been described in SFG rickettsiae [9].

The epidemiological implications of a rickettsial organism, different from *R. conorii*, which infects Spanish dog ticks should be considered, because this

organism could affect dogs and/or humans in several ways such as protecting them from infection by other pathogenic rickettsiae, or producing some type of clinical or subclinical infection.

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